THE UPTAKE AND UTILIZATION OF PHOSPHATE IONS FROM SEA WATER BY THE AMERICAN OYSTER, CRASSOSTREA VIRGINICA (GMEL.) [1]

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Phosphorus is of physiological importance to mollusks not only in their carbohydrate metabolism and energy transfer systems but also in shell deposition. Both Manigault (1939) and Bevelander and Benzer (1948) have associated shell deposition with phosphatase activity, and the latter also suggested that the first material to be deposited is calcium phosphate, which is changed into calcium carbonate in the presence of a phosphatase. Love and Frommhagen (1953) have found further evidence that calcium phosphate is the precursor of calcium carbonate in *Mactra* (*Spisula*) *solidissima*. Bevelander (1952) exposed several species of mollusks to $P^{32}O_4$ ions in sea water and found in radioautographs that P^{32} was localized on the inner surface of the mantle, below the surface epithelium. Since aqueous methods were used in the preparation of the tissues, the P^{32} shown by his radioautographs was only that fraction which had entered water-insoluble combinations.

Two sources of phosphorus are potentially available to marine, ciliary feeding mollusks: that combined in suspended particulate matter, such as plankton and detritus, and that in solution in sea water in various chemical combinations. The latter may be divided into phosphate ions in equilibrium with various cations and dissolved organic compounds which include phosphorus in their makeup.

Ronkin (1950) has shown that $P^{32}O_4$ ions are absorbed by the excised gill of *Mytilus edulis,* and he suggests that some of this phosphorus is used in the production of adenosine triphosphate by the gill, to be used in maintaining the extensive ciliary action there. It has been further shown (Pomeroy and Haskin, 1951) that phosphate ions are absorbed by the oyster from sea water principally through the gills, and that these ions appear rapidly in the blood and eventually in other tissues. This report gives further information on the uptake and distribution of phosphorus in the oyster, the absorptive mechanism, and the importance of the direct utilization of ions from the surrounding water.

Materials and Methods

Phosphorus analyses on sea water were performed by the method of Redfield *et al.* (1937), modified for the use of a photometer. Phosphorus analyses on tissues were made by wet-ashing them in sulfuric and nitric acid, driving off the excess nitric acid by slow heating, carbonization of the organic matter in the remaining sulfuric

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acid, oxidation with 30 per cent hydrogen peroxide, and proceeding as with sea water samples. All tissues were collected and ashed in duplicate. Aliquots from the wet-ashed tissue samples were evaporated to dryness on planchets of uniform area and counts made of their radioactivity using a scaler and counting tube with an end-window density of 1.4 mg./cm.^{2}. Samples were sufficiently small and dilute to prevent appreciable self-absorption. This was verified by counts of a suitable graduated series of sample dilutions.

Radioautographs were made by two of the methods of Holt and Warren (1950), one of which involved quick freezing and vacuum dehydration while the other involved fixation in neutral alcohol-formalin fixative. In addition, radioautographs were prepared of frozen gross serial sections of whole oysters. The latter were particularly useful in studying phosphorus distribution. Their preparation involved quick-freezing the whole oyster, rapidly preparing gross sections three to four millimeters in thickness of the frozen specimen, and clamping them against cold nuclear track plates. The sections were kept frozen throughout the cutting, clamping, and exposure periods. Eastman NTB nuclear track plates with a ten-micron emulsion proved satisfactory, even when exposure involved prolonged storage in a freezer. The simplicity of the process permitted the preparation of radioautographs of serial gross sections of a number of oysters with a minimum of time and equipment. Since the sections remained frozen throughout, there was no loss or movement of soluble radioisotopes.

The oysters used were Delaware Bay market-size oysters. Nearly all the oysters were from a single lot which was stored in trays in the Bay. Small groups were brought to the laboratory by car periodically. There they were kept in a cold room, if not needed immediately. When stored in a cold room, they were placed in sea water at 20° C. for 24 hours before use, and were then transferred to fresh sea water. The responses of stored oysters were normal when compared with freshly collected ones.

All oysters were scrubbed with a stiff brush and washed in running water to remove sand, loose shell fragments, and attached organisms. Measurements of the radioactivity of the outer surface of oyster shells at the end of experiments indicated that there was little adsorption of phosphate ions or accumulation of radioactive phosphorus by such microflora as might have remained attached.

Each of the experiments described represents one of several replications made at somewhat varied initial phosphate concentrations.

Observations

1. Uptake of phosphate by oysters

Groups of six oysters were placed in individual battery jars, each with one liter of water containing 40 micrograms of phosphate phosphorus, to which 20 microcuries of P^{32} as H_3PO_4 had been added. The addition of the radioisotope did not increase the concentration of phosphate ions in the sea water significantly. A constant temperature of 18° C. was maintained. After various time intervals a group of six was removed for assay of the tissues. Duplicate samples were taken of several tissues. All oysters were observed to establish the fact that they were open while in the labelled sea water. The results are shown in Figure 1. The apparent

drop in the mean specific activity of most tissues at 64 hours as compared with 16 hours is not significant. Individual variation was so great that uptake rates are only approximated by this method. This is particularly true in the case of the digestive gland, with a very low and variable uptake rate. It appears, however, that a steady state is reached at about 16 hours.

Several oysters which did not open while in the labelled sea water were also assayed. None showed any radioactivity in the tissues, although in most cases the

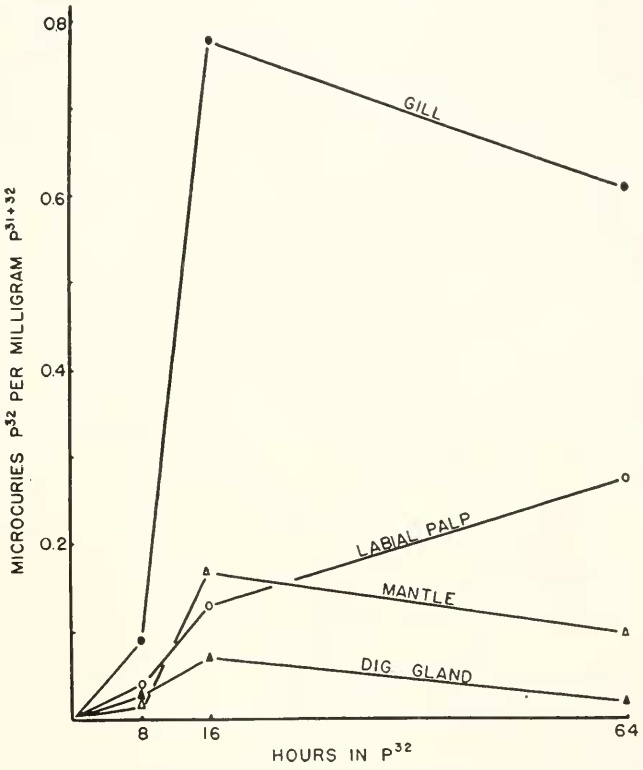

FIGURE 1. Uptake of phosphate ions labelled with P^{32} from sea water by groups of oysters. See text for description.

pallial border was exposed, for the shells had been chipped to facilitate opening them when radioactive.

2. Uptake of phosphate ions by excised oyster tissues

Excised tissues from three oysters were placed in P^{32}-labelled sea water which was aerated and maintained at 18° C. in a water bath. The pieces of tissue were about one $cm.^2$ in size. The amount of exposed cut surface varied, being comparatively little in the case of gills and great in the digestive gland and adductor muscle. After time intervals comparable to those in the preceding study, duplicate samples

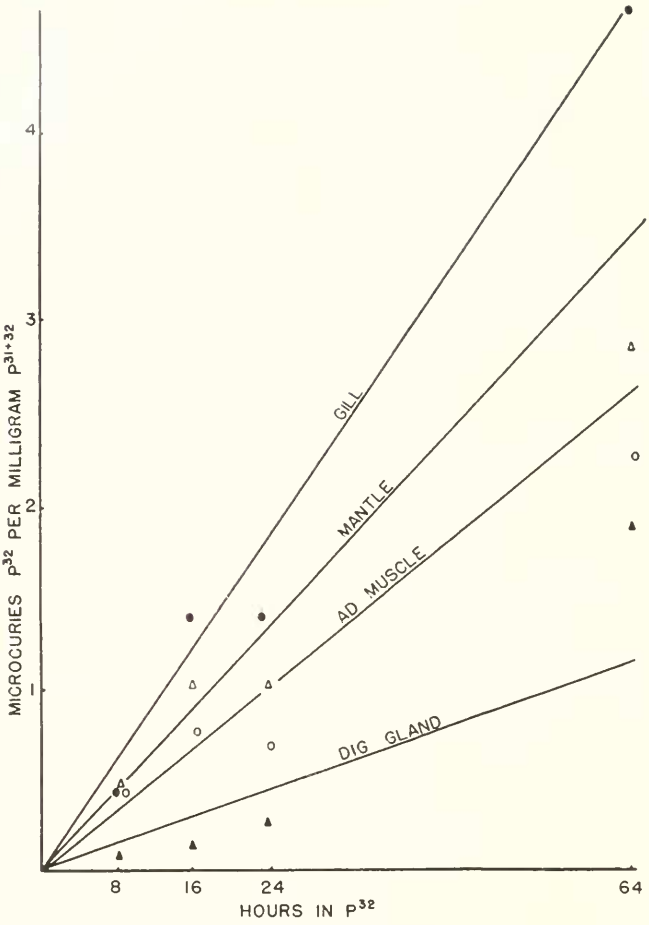

FIGURE 2. Uptake of phosphate ions labelled with P^{32} from sea water by groups of excised tissues of oysters. Gill, ●; mantle, △; adductor muscle, ○; digestive gland, ▲. See text for description.

of each tissue were removed and assayed. Figure 2 shows the results, with computed lines of best fit. The relationship of uptake to time is essentially linear.

3. Labelled phosphorus in the blood

Oysters were placed in labelled sea water as in the first experiment. After various time intervals samples of blood were removed from the heart and assayed. The results are shown in Table I. Each value is the mean of duplicate samples of six oysters. The total blood phosphorus varied in different individuals from eight to 30 micrograms per milliliter. However, there was a labile fraction which was relatively consistent in terms of per cent of total phosphorus.

TABLE I

Mean uptake of phosphate ions by the blood of oysters in sea water labelled with P^{32} at 18° C., expressed as per cent of total blood phosphorus

Hours in P^{32}	1	2	4	8	12
% of blood phosphorus labelled	0.03	0.05	0.08	0.08	0.09

4. Excretion of phosphorus by oysters

Several oysters were placed in individual aerated battery jars containing filtered sea water maintained at 18° C. The water was replaced at two- to four-day intervals for 33 days. Phosphorus determinations were made before and after the water was used. The average daily phosphorus excretion is shown in Table II. Each entry represents the mean daily value for the days the water was in use. The oysters had been in dry cold storage prior to the experiment, and probably voided some accumulated wastes at the beginning. After a week there was little change in the rate of phosphorus excretion. The oysters received no food other than the dissolved or exceedingly small materials which passed through the cotton filter.

TABLE II

Excretion of phosphorus by three oysters, expressed in micrograms of phosphorus excreted per 24 hours

Days:	2	5	7	10	12	16	20	26	33
No. 1	620	100	30	50	40	40	40	20	20
No. 2	560	110	100	80	50	40	40	20	20
No. 3	500	130	100	50	50	40	30	20	20

5. Radioautographs of phosphorus distribution

Oysters were placed in labelled sea water as in the first experiment, after which radioautographs were prepared of several tissues and of gross serial sections of whole oysters. After four hours in P^{32}-labelled sea water the gills showed P^{32} to be generally distributed in them. Other tissues showed no activity, except one specimen which showed activity in one of the peripheral blood vessels of the mantle. After 12 hours in P^{32} most tissues produced radioautographs of nearly uniform density. After 24 hours radioautographs were still uniform though more intensely darkened.

DISCUSSION AND CONCLUSIONS

After periods of one to four hours of exposure to $P^{32}O_4$-labelled sea water, P^{32} is detected in oysters only in the gills and blood taken from the heart, indicating that most, if not all, phosphate ions absorbed directly from sea water enter through the gills and reach other tissues via the blood. After 12 hours only about 0.1 per cent of the total blood phosphorus is derived directly from dissolved phosphate ions.

This small labile fraction may be one of the principal limitations of uptake rate for the organism as a whole. However, additional factors may limit the rate of uptake of phosphate ions by individual tissues. In repeated experiments with excised tissues, their respective uptake rates always fell in the same order. This suggests that each has a characteristic relative uptake rate.

The observation that radioautographs of the tissues of oysters which had been in sea water containing $P^{32}O_4$ ions were uniformly darkened suggests to us that most of the phosphate ions remain in the tissue fluids for the first 24 hours after absorption. At least, there is not enough concentration of the labelled ions in any tissue to cause a noticeably greater darkening of any portion of the radioautographs. The radioautographs of Bevelander (1952), however, show that in some mollusks a fraction is incorporated into insoluble materials within this period. If this is also true in the case of oysters, and probably it is, then the amount of absorbed phosphorus converted to water-insoluble compounds in 24 hours must represent a small fraction of the total absorbed phosphorus.

If in experiment (1) we take 0.1 microcurie per milligram of phosphorus as the approximate mean specific activity of the whole oyster after 24 hours in labelled sea water and assume (on the basis of many analyses) that the average oyster weighs 10 grams and contains 20 milligrams of phosphorus, then the oysters in this experiment absorbed four micrograms of phosphorus in 24 hours. The basal phosphorus loss through excretion is 20 micrograms in 24 hours. The amount of phosphorus obtained from ions in sea water in this case is about 20 per cent of the basic requirement of the oyster. However, in other experiments with different groups of oysters the fraction of the basal requirement obtained from ions in sea water varied from five to 50 per cent. These values must be considered to be only approximations.

The levelling-off of specific activity in the intact tissues, as contrasted with the continued accumulation in excised tissues, suggests that in the former the uptake is balanced by losses from the tissues. The physiological significance of this steady-state cannot be judged from available information.

Leenhardt's (1926) studies of *Gryphaea* (*Crassostrea*) *angulata* indicate that the gill circulation is by-passed by the principal blood vessels, but that there is a small exchange at all times between the gill circulation and the general circulation. If this is also true of *C. virginica*, it may be one factor limiting the utilization of dissolved phosphate. However, the gills, which obtain phosphate ions from the water without mediation by the blood, also reach a steady-state of uptake after about 16 hours. Therefore, some limiting factor other than the rate of circulation and the amount of phosphorus carried by the blood would seem to exist.

Since the gills take up relatively greater amounts of phosphate from the water and obtain it directly, it is interesting to speculate that the gills may be autonomous to some degree. They may obtain a substantial part of their nutritional needs directly by absorption and local phagocytosis.

The observation that no P^{32} entered oysters which remained closed during their exposure to labelled sea water, even when the margins of their shells were imperfect, shows that the pallial curtains are capable of completely sealing off the mantle cavity from the external medium. It further verifies the observation that the tissues of the pallial curtain do not play a large role in the direct absorption of phosphate ions from sea water.

A high degree of physiological variability was noted in the oysters used in this work, although every effort was made to use oysters from a single local population and of one size class for any given experiment. Great variability was also found by Wilbur and Jodrey (1952) in the shell deposition process. Space limitations and the large volumes of radioactive sea water involved made the use of larger groups of oysters impractical.

It is probable that other dissolved materials are absorbed through the pathway which has been demonstrated for phosphate ions. Preliminary studies with Ca^{45} indicate that relatively greater amounts of calcium are absorbed by the gills and carried in the blood. This may be the principal source of calcium for shell deposition.

Summary

1. Phosphate ions in sea water are absorbed by oysters, principally by the gill, and are carried by the blood to all parts of the organism.

2. Directly absorbed phosphate ions are incorporated into the tissues of adult oysters slowly, most remaining in water-soluble forms 24 hours after absorption.

3. Significant amounts of phosphorus may be obtained by oysters through the direct absorption of phosphate ions from sea water.

4. After 12 hours in $P^{32}O_4$, directly absorbed phosphate ions make up less than 0.1 per cent of the total blood phosphorus. This may be one of the several factors which limit the utilization of phosphorus from sea water.

LITERATURE CITED

Bevelander, Gerrit, 1952. Calcification in molluscs. III. Intake and deposition of Ca^{45} and P^{32} in relation to shell formation. *Biol Bull.*, **102**: 9–15.

Bevelander, Gerrit, and P. Benzer, 1948. Calcification in marine molluscs. *Biol. Bull.*, **94**: 176–183.

Holt, M. W., and Shields Warren, 1950. A radioautographic method for detailed localization of radioactive isotopes in tissues without isotope loss. *Proc. Soc. Exp. Biol. Med.*, **73**: 545–549.

Leenhardt, Henry, 1926. Quelques études sur *Gryphaea angulata* (huitre du Portugal). *Ann. Inst. Oceanog.*, **3**: 1–90.

Love, Robert, and L. H. Frommhagen, 1953. Histochemical studies on the clam, *Mactra solidissima*. *Proc. Soc. Exp. Biol. Med.*, **83**: 838–844.

Manigault, P., 1939. Récherches sur le calcaire chez les mollusques. Phosphatase et précipitation calcique. Histochimie du calcium. *Ann. Inst. Oceanog.*, **18**: 331–426.

Pomeroy, L. R., and H. H. Haskin, 1951. The uptake of radioactive phosphate ions by the oyster, *Crassostrea virginica* Gmelin, and their distribution in the tissues. *Anat. Rec.*, **111**: 15.

Redfield, A. C., H. P. Smith and B. H. Ketchum, 1937. The cycle of organic phosphorus in the Gulf of Maine. *Biol. Bull.*, **73**: 421–442.

Ronkin, R. R., 1950. The uptake of radioactive phosphate by the excised gill of the mussel, *Mytilus edulis*. *J. Cell. Comp. Physiol.*, **35**: 241–250.

Wilbur, K. M., and Louise Jodrey, 1952. Studies on shell formation. I. Measurement of the rate of shell formation using Ca^{45}. *Biol. Bull.*, **103**: 269–276.